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ON THE BIOLOGIC PROPERTIES OF PATHOGENIC MOLDS *

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The biologic properties of the higher fungi are almost totally ignored in most works dealing with mycology and particularly bacteriology, despite the fact that their proteolytic ferments, at least, have been made the object of numerous studies. Attempts to classify these organisms along these lines have, to date, failed; yet these properties, although not of practical value, are at least of value from a scientific point of view.

It might be well to remark here, that after 18 months' study of the growths of ringworms, obtained from Dr. Sabouraud, on various culture mediums of American made ingredients, we have, with other investigators, come to the conclusion that for the present, recognition of any of these organisms depends on their characteristics developed on mediums made with the French ingredients and methods used by Dr. Sabouraud. If Dr. Sabouraud's studies, descriptions, and methods, all of which depend on culture mediums, whose ingredients are difficult to obtain in America, are to be accepted finally as a basis for the classification of the pathogenic molds, then it appears that only by studies along these lines can we arrive at a definite conception of the pathogenic ringworm flora of America, unless the all too difficult problem of French products or their exact chemical nature and p_H be solved by the use of American produced substances.

So far as I have been able to determine, the studies undertaken in this paper have been limited to few fungi and generally to the most important of their ferments, namely, gelatinase. Is this proteolytic property possessed by all of the higher vegetable parasites, and what is the relative activity of that possessed by each of them. Is this ferment soluble or insoluble in all cases, and to what extent?

In 1895, MacFadyen[1] definitely showed that the ringworm fungus produced a ferment capable of digesting gelatine. Although he worked

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[1] Jour. Path. & Bacteriol., 1889, 33, p. 176.

with fresh specimens of the tricophyton, he made no note of the varieties used. However, MacFadyen not only determined that the tricophytons he used possessed proteolytic powers, but that the enzyme was soluble and given off by the fungus as was indicated by the digestion of fresh gelatine when liquefied fungus-free digested gelatine was added to it. He further demonstrated that a temperature of 100° C. for 2 minutes destroyed this ferment, and that acids retarded, whereas alkalis accelerated, its action.

Roberts [2] showed that even 6-year old specimens of fungi retained this property, and came to the following conclusions:

1. That the active agent is contained in the mycelium itself and is not formed outside the body of the parasite by secondary changes in the medium.
2. When the fungus has been preserved in a perfectly dry state, that this active principle can retain its activity in a dormant state for a period of 6 years.
3. That the activity of the agent is independent of the life of the fungus.
4. That small amounts of the active agent can digest comparativelv large amounts of gelatine.
5. That this active agent is in the nature of a soluble ferment.
6. That the activity is destroyed by heat at a temperature of 85 C. for 2 minutes.

Like MacFadyen, Roberts drew his conclusions from a study of a limited number of these organisms. Most of his work remains exact. It is interesting to note that as a result of his studies as well as those of Ruffi, the proteolytic property of these parasites have no relation to their virulence since it is present in dead fungi also. And the latter fact is true, not only for the limited number of organisms studied by him, but likewise for all of the organisms studied in this paper.

This study has for its objects:

1. A study of the proteolytic ferments of 26 pathogenic fungi.
2. A study of the amylotic ferments.
3. Their action on various sugars and on litmus.
4. A study of indol production.
5. A study of the toxins of several species.

PROTEOLYTIC FERMENTS

The following table gives the organisms studied and their comparative proteolytic properties in 10 cc of 10% gelatin. Plus 3 is used to indicate complete liquefaction in 24 hours; plus 2, a moderate liquefaction for the same period of time; plus 1, a slight liquefaction; and —, none at all. Since all these organisms are particularly sensitive to changes in heat, light, moisture, etc., the experiments were conducted under as uniform conditions as possible.

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[2] Brit. Med. Jour., 1899, 24, p. 13.

ORGANISMS STUDIED AND THEIR COMPARATIVE PROTEOLYTIC PROPERTIES IN 10 CC OF 10% GELATIN

Organism	Liquefaction in 24 Hours
Tricophyton niveum radians	Plus 3
Tricophyton vinosum	Plus 3
Microsporon pubescens	Plus 3
Achorion quinckeanum	Plus 3
Tricophyton violaceum	Plus 2
Tricophyton fumatum	Plus 2
Tricophyton lacticolor	Plus 2
Sporotrichum beurmanii	Plus 2
Sporotrichum schenkii	Plus 2
Tricophyton crateriform	Plus 1
Tricophyton acuminatum	Plus 1
Tricophyton sulphureum	Plus 1
Tricophyton exsiccatum	Plus 1
Tricophyton cerebriform	Plus 1
Tricophyton plicatile	Plus 1
Tricophyton gypseum asteroides	Plus 1
Tricophyton granulosum	Plus 1
Microsporon lanosum	Plus 1
Tricophyton roseaceum	—
Microsporon audouini	—
Microsporon fulvum	—
Sporotrichum gougeroti	—
Actinomyces bovis	—
Achorion schoenleinii	—
Achorion gallinae	—

The ring-worm fungi in this group, however, showed liquefaction at a later stage.

SOLUBILITY OF THE FERMENT

Truffi, as well as Roberts, showed that liquefied gelatine was able to hold and transmit this proteolytic body, but this is certainly not true of all the fungi. All the organisms in this study did not exhibit this property when filtered liquefied gelatine was used, and we might therefore conclude that gelatinase in them is not a soluble one. Fresh gelatine in contact for 72 hours with liquefied gelatine obtained with Microspora audouinii and lanosum, Tricophyton lacticolor, Achorion quinckeanum and Sporotrichum (beurmanii and schenkii) showed no liquefaction at all with the parasites just noted.

Second, this special enzymotic power, as present in liquefied gelatine, varies definitely in strength with the organism. Whereas a certain number will liquefy the fresh gelatine in 24 hours when the same amounts of liquefied material are used (vinosum, pubescens, niveum radians), others require 48 hours to complete it (plicale, cerebriform, sulphureum).

Summary.—Gelatinase is a common property of the higher vegetable parasites, and varies with the organism, but not so much because of the rapidity with which that organism grows, since Tricophyton gypseum asteroides is a "quick grower," in mycologic parlance, and yet caused but slight liquefaction at the end of 24 hours. It is a question of quantity or quality of gelatinase, or both. Furthermore, the solubility of this ferment varies with the organism.

AMYLOLYTIC FERMENTS

Both MacFadyen and Roberts, as well as Bodin,[3] working with Achorion gypseum, were unable to demonstrate an Amylolytic ferment. We were unable to detect sugar with Benedict's solution at any time during the 2 months of their growth in a starch-water medium. They grow quite well in 3 and 5% starch solutions, indicating that they can assimilate the larger starch molecule without previous hydrolytic changes.

SUGAR FERMENTATION AND LITMUS REACTION

For this study a Russell's modified double sugar medium prepared with lactose and glucose, as usual, was used, and a series of tubes each prepared with one only of the following: lactose, glucose, maltose, saccharose, dextrin, and levulose, and with end result adapted for fungi.

The effects of each fungus on one double sugar and one single sugar medium were studied for color changes and gas production.

It may be noted that all the fungi studied grew quite well on these mediums, but most lacked the characterization given them by Sabouraud's concentrated and proof mediums.

None of the organisms listed below altered litmus or caused fermentation of any of the sugars: Tricophyton, acuminatum, crateriform, violaceum, fumatum, sulphureum, exsiccatum, cerebriform, plicatile, asteroides, granulosum, lacticolor, radians, rosaceum, vinosum, ochraceum; Microsporon, audouini, pubescens, fulvum, lanosum; Achorion, schoenleinii, gallinae, quinckeanum, gypseum.

These organisms were also grown on the same medium without peptone, but did not, under these circumstances, ferment any of the sugars used.

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[3] Ann. de Dermat., 1907, 8, p. 585.

INDOL PRODUCTION

Böhme's technic[4] was used. The organisms were planted in sheep serum broth. All the implants did not grow, but in none of those that grew, were we able to detect indol.

TOXIN PRODUCTION BY PATHOGENIC FUNGI

Cutaneous affections caused by the higher vegetable organisms are ordinarily not associated with systemic disturbances indicative of intoxication, and few among the numerous investigators have been able to demonstrate the presence of toxic substances elaborated by them.

As the work of Plato, Block, and Massini[5] has shown, the dermatophytous do produce toxins. Further, toxic soluble substances have been shown to be elaborated by Oidium albicans (Charrin, Ostrowsky, Roger, Concetti quoted by Brumpt), by Discomyces and some species of Aspergillus. That they really have a toxic influence is shown by the destruction of bone by the ray fungus, the abscesses in sporotrichosis, blastomycosis, kerion and folliculitis agminata.

In our work 5 organisms were studied: Tricophyton acuminatum and gypseum-asteroides; microsporon audouini, sporotrichum beurmanii and achorion schoenleinii.

These fungi were cultivated in a proof-medium with bouillon as the base, and sealed to prevent evaporation. The tricophytons and sporotrichum were grown for 8 weeks; the microsporon and achorion, for 14 weeks. At the end of these periods, these were filtered, after thoroughly breaking up the growth, first through sterile filter paper, then through a sterile kitasato candle. A control flask of this medium was filtered in the same manner.

Eight ounce guinea-pigs were injected intraperitoneally with 2 c c of the control and 2 c c of each filtered culture. The pigs apparently suffered no untoward reaction. One week later, 4 c c of each medium were used. The microsporon, sporotrichum, and the control gave negative results, whereas the pigs injected with the tricophytons and achorion died. That injected with T. acuminatum succumbed in 24 hours; that with T. gyp. asteroides, 10 days later, and that with achorion, 48 hours later.

After death there was found mainly a marked suprarenal vascular injection. The appearance resembled that produced by the intraperitoneal injection of diphtheria toxin. Cultures made from the peritoneal fluid remained negative.

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[4] J. Am. Med. Assoc., 1921, 77, p. 959.

[5] Cited by Sabouraud, Les Teignes, 1902, p. 735 and 736.

Sabouraud[6] states "That many writers have inoculated the elaborated products of cultures, but that the resultant effects were due to the toxic action of the peptones." Inasmuch as the control pig suffered no ill effects, it seems natural to conclude that the tricophytons and the achorion schoenleinii do elaborate toxic products.

CONCLUSIONS

A proteolytic ferment is common to all the pathogenic molds studied, but its solubility varies with the organism.

They possess no amylotic properties.

They do not ferment saccharose, dextrin, glucose, levulose, maltose or lactose, and they produce no acids or bases.

The tricophytons (Tricophytons acuminatum and gypseum-asteroides) and Achorion schoenleinii elaborate toxins fatal to guinea-pigs.

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[6] Les Teignes, 1902.